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***RESEARCH ARTICLE*.....**

Fish visceral protease – an alternative source for recovery of silver from waste X ray photographic films

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ABSTRACT..... Fish processing in India generates enormous amount of solid fish waste and causes various kinds of pollutions. Actually these wastes are used for the production of a variety of value added products such as enzymes mainly protease. Protease enzyme has diverse applications in a wide variety of industries such as detergent, food, pharmaceutical and leather industries, peptide synthesis and for the recovery of silver from used X-ray films. In this paper, *Labeo rohita* fish visceral waste was collected from Mettur Dam, Tamil Nadu. A crude homogenate was prepared and the protease activity was confirmed by zymography. After that an experiment was carried out with the isolated crude protease to prove its efficiency to recover silver from waste X ray photographic films.

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INTRODUCTION.....

Silver is one of the precious and noble metals used in large quantities for many purposes, particularly in the photographic industry. The waste X-ray/photographic films containing black metallic silver spread in gelatin are very good source for silver recovery compared to other types of film. The amount of silver in the X-ray film varies between 1.5 and 2.0 per cent (w/w). It has been reported that 25 per cent of the world's silver needs are supplied by recycling out of which 75 per cent is obtained from photographic waste. With an increasing demand for silver in the world, recent attention is focused on X-ray/photographic films as one of the secondary sources of silver owing to the considerable amount of silver present in them (Shankar *et al.*, 2010).

The various studies carried out for the recovery of silver from photographic / X – ray film wastes are burning of films directly, oxidation of the metallic silver following electrolysis, stripping of gelatin layer using different chemicals and enzymatic hydrolysis of gelatin – silver (Nakiboglu *et al.*, 2003). Conventionally silver was being recovered by burning the films directly which generates undesirable foul smell and causes environmental pollution. Furthermore, base film made of polyester film on which emulsion of silver and gelatin is coated cannot be recovered. Since the emulsion layer on X-ray film contains silver and gelatin, it is possible to break down the gelatin layer using proteases and release the silver. The enzymatic hydrolysis of the gelatin layers on the X-ray film enables not only the recovery of the silver but

also the polyester base which can be recycled.

On the other hand, enzyme from microbial source breaks the gelatin layer embedded with silver in films creating pollution free stripping. Though the enzymatic method is slow, it is cost effective too (Choudhary, 2013). In this study a trial has been made to use fish visceral enzymes, that might be an alternative source for the recovery of silver from waste X ray photographic films. Generally fish processing operations produce waste in the solid form like fish carcasses, viscera, skin and heads and liquid form like washing and cleaning water discharges, blood water from drained fish storage tanks, brine (Michail *et al*., 2006). However, some of the by-products are utilized, the main bulk is dumped to waste creating both disposal and pollution problems (Norziah *et al.*, 2009). Moreover, the viscera are a good source of digestive enzymes that may have some unique properties of interest to both basic and industrial applications (Fuchise *et al*., 2009). This waste is regarded as one of the richest sources of proteolytic enzymes. Proteases have diverse applications in a wide variety of industries such as detergent, food, pharmaceutical and leather industries, peptide synthesis and for the recovery of silver from used X-ray films. Proteases are mainly derived from animal, plant and microbial sources (Nasri *et al*., 2011). This study therefore is an effort to minimize pollution caused due to ignorant generation of such wastes and at the same time utilize them for the benefit of mankind.

RESEARCH METHODS.....

Labeo rohita viscera :

The viscera of the fish *Labeo rohita* were collected from the local market in Mettur Dam, Tamil Nadu, India. The visceral samples were kept in ice and transported to the place of work within one hour. It was then washed with distilled water and stored in sealed plastic bags at -20°C.

Preparations of crude enzyme extract (El Hadj *et al.*, 2009) :

The collected viscera were thawed for about 2 hours at room temperature and then homogenized with 200 ml of homogenization buffer (10 mM Tris HCl, pH 8.0). The homogenate was centrifuged at 8500×g for 30 min at 4°C. The pellet was discarded and the supernatant (50 ml) was collected and used as the crude protease extract.

Protease assay :

Protease activity was assayed by the Anson method with some modifications. The crude enzyme solution (1 ml) was mixed with 5.0 ml of substrate (0.65% casein in 25 mM Tris - HCl buffer, pH 8.0) at room temperature for 30 min. After incubation, TCA (110 mM) was added to attenuate the reaction. This mixture was allowed to incubate for 30 min at room temperature and filtered to remove the precipitate. Then 2 ml of the filtrate was taken in a test tube and 1 ml of the Folin Ciocalteu's reagent was added. The absorbance was measured at 660 nm. A standard curve was generated using solutions of 0.2 mg/ml tyrosine. One unit will hydrolyze casein to produce colour equivalent to 1.0 μmole (181 μg) of tyrosine per minute at pH 8.0 at 37°C.

Protein determination :

Protein concentration was estimated by Lowry *et al*. (1951) using Bovine Serum Albumin as standard.

Testing of protease on gelatin (Shankar *et al*., 2010 and Ramanujam, 1974) :

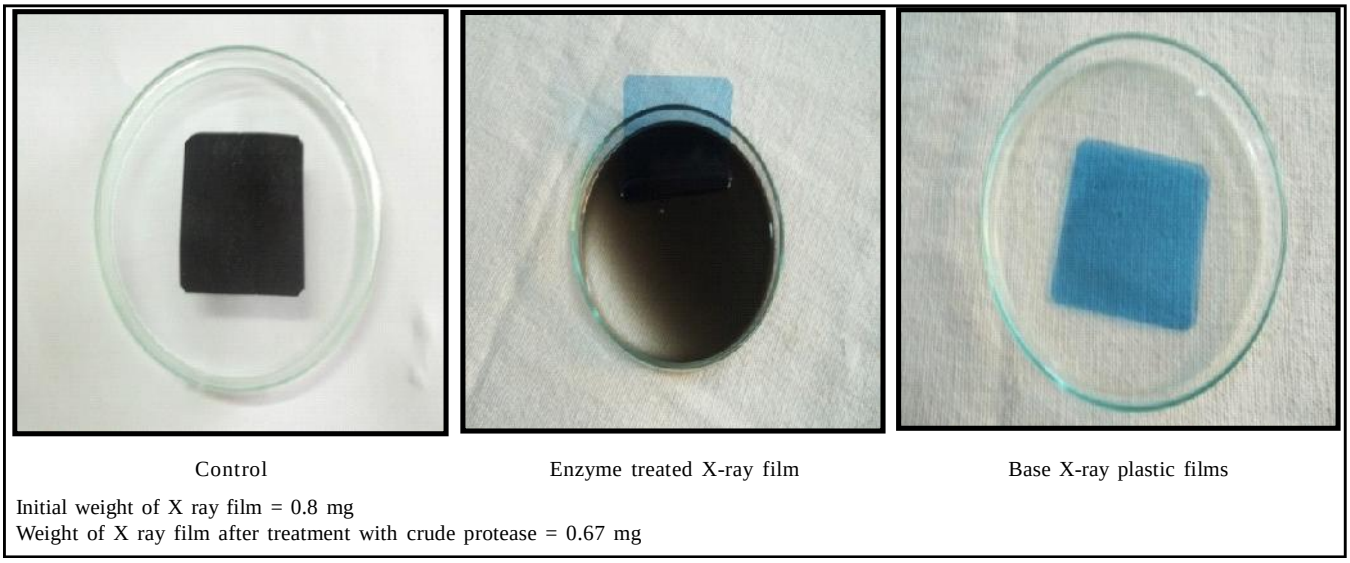
The waste X-ray film was washed with distilled water and wiped with cotton impregnated with ethanol. The film was dried in an oven at 40^0C, for 30 min. Cut the one g of X-ray film into 2 x 2 cm pieces and incubated with 10 ml of crude protease (such that the film is completely immersed in the solution) at 40^0C, pH 8.0 in a water bath with continuous shaking. Turbidity of the reaction mixture was observed. Progress of hydrolysis was monitored. The presence of silver was checked by qualitative tests.

RESEARCH FINDINGS AND ANALYSIS.....

Alkaline proteases play a crucial role in the bioprocessing of used X-ray films or photographic films for silver recovery (Gupta *et al*., 2002).

"Plate shows the X-ray films treated with the crude fish visceral protease".

It is shown in Plate 1 that treatment of X-ray films with protease resulted in the sliver bound with gelatin being stripped off into the reaction mixture and the clean plastic film being recovered. A loss in weight of 0.67 mg after the treatment was also observed as compared to the initial weight of the film which was 0.8 mg. The



Control | Enzyme treated X-ray film | Base X-ray plastic films

Initial weight of X ray film = 0.8 mg
Weight of X ray film after treatment with crude protease = 0.67 mg

Plate 1 : Action of fish visceral protease on X-ray films

presence of silver in the hydrolysate was confirmed by a qualitative test.

The above results are supported by the observations of Shankar *et al*. (2010) who reported that alkaline protease from *Conidiobolus coronatus* investigated for enzymatic hydrolysis of gelatin from waste X-ray films, showed the complete removal of gelatin and silver leaving the polyester film clean and the silver recovered in the hydrolysate, both of which could be reused. A report by Ramakrishna *et al*. (2010) also observed that, *Bacillus subtilis*, isolated from slaughter house soil could degrade the gelatinous coating of X-ray films. Treatment of X-ray films with protease results in the release of silver, as it binds with gelatin which is being stripped off into the reaction mixture to get a clean plastic film.

Conclusion :

The present study aims at isolating proteases from the viscera of *Labeo rohita,* a type of fish which is abundantly consumed by populations of Salem. The fish species is readily available in the local markets of Mettur Dam, Tamil Nadu, India. The viscera are a waste and are not used as a source of food and thus are rejected. This leads to a large accumulation of wastes in commercial places contributing to pollution of water and soil resources. Hence, the objectives of the study was to prepare the crude protease extract from visceral organ wastes of *Labeo rohita* fish, and subjected to qualitative application to confirm its ability to hydrolyze gelatin on the waste X ray photographic films.

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